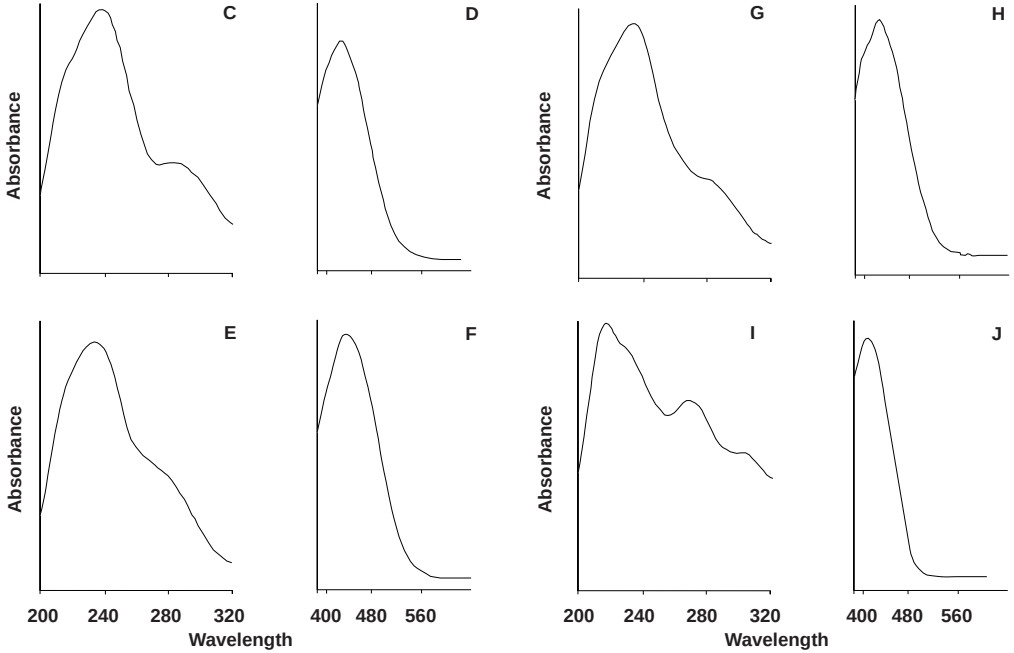

Section 4
References

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7. Thormann, W., Caslavska, J., Molteni, S. and Chmelik, J., Capillary isoelectric focusing with electroosmotic zone displacement and on-column multichannel detection, *J. Chromatogr.*, **589**, 321-327 (1992).

Section 3
Product Information

Catalog Number	Product Description
148-2100	**BioMark Synthetic pI Marker Kit**
148-2101	**BioMark Synthetic pI Markers,** pI 5.3, 6.4, 7.4, 8.4, 10.4, 200 µl
148-2102	**BioMark Synthetic pI Marker,** pI 5.3, 200 µl
148-2103	**BioMark Synthetic pI Marker,** pI 6.2, 200 µl
148-2104	**BioMark Synthetic pI Marker,** pI 6.4, 200 µl
148-2105	**BioMark Synthetic pI Marker,** pI 6.5, 200 µl
148-2106	**BioMark Synthetic pI Marker,** pI 6.6, 200 µl
148-2107	**BioMark Synthetic pI Marker,** pI 7.0, 200 µl
148-2108	**BioMark Synthetic pI Marker,** pI 7.2, 200 µl

Catalog Number	Product Description
148-2109	**BioMark Synthetic pI Marker,** pI 7.4, 200 µl
148-2110	**BioMark Synthetic pI Marker,** pI 7.5, 200 µl
148-2111	**BioMark Synthetic pI Marker,** pI 7.7, 200 µl
148-2112	**BioMark Synthetic pI Marker,** pI 7.9, 200 µl
148-2113	**BioMark Synthetic pI Marker,** pI 8.4, 200 µl
148-2114	**BioMark Synthetic pI Marker,** pI 8.5, 200 µl
148-2115	**BioMark Synthetic pI Marker,** pI 8.6, 200 µl
148-2116	**BioMark Synthetic pI Marker,** pI 10.1, 200 µl
148-2117	**BioMark Synthetic pI Marker,** pI 10.4, 200 µl
148-5028	**CIEF Catholyte,** sodium hydroxide, 60 ml x 4

Catalog Number	Product Description
148-5029	**CIEF Anolyte,** phosphoric acid, 60 ml x 4
148-5030	**CIEF Mobilizer,** 60 ml x 4
148-5031	**CIEF BioLyte Ampholyte,** pH 3-10, 2%, 60 ml
148-3070	**BioCAP™ LPA coated capillary,** 100 cm x 50 µm ID x 360 µm OD, window 60 cm from end

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547
4105015 Rev B

BioMark™ Synthetic pI Markers for Capillary Isoelectric Focusing

Products shipped at room temperature

For research use only

Store at 4 °C

Catalog Numbers 148-2100 through 148-2117

BIO-RAD

Section 1
Introduction

Capillary isoelectric focusing (CIEF) is a high-resolution separation technique for separation of proteins based on their isoelectric points. The technique provides separations similar to those obtained with conventional gel isoelectric focusing, but is easier to perform, can be fully automated, and has the potential for providing quantitative information. For accurate determination of protein isoelectric points and for achieving best reproducibility, the use of isoelectric point (pI) markers is suggested. Pure proteins of known pI values have been used for this purpose, but in many cases it is difficult to obtain highly pure preparations of the desired marker proteins and the proteins may degrade with storage and use, generating interfering degradation products and variable detector response.

The BioMark synthetic pI markers are low molecular weight ampholytes which possess well-defined isoelectric points and strong UV absorbance.[1] They can be used as external standards for calibration of the CE system and estimation of protein isoelectric points, and can be added to a protein sample as internal standards for accurate determination or confirmation of protein pI values. The markers are very stable and should exhibit no degradation under conditions of normal use and storage. These highly-colored compounds are easily detected at the 280 nm wavelength typically used for CIEF of proteins, even when diluted 100-fold in the sample + ampholyte solution. In addition, their absorption in the visible region can be used to confirm peak identity when the markers are used as internal standards in complex protein mixtures. All of the markers exhibit maxima in the 300–400 nm spectral range.

The instructions in this publication include information for the use of the BioMark Synthetic pI Marker Kit and for use of the BioMark Synthetic pI Markers purchased individually.

Specifications

Contents	Approximately 1 mg/ml concentration in deionized water
Volume	200 µl
Storage	1 year at 4 °C
Applications per vial	at least 200

Section 2
Instructions

The BioMark synthetic pI markers can be used for any of the capillary IEF techniques which have been described, including ion addition

mobilization,[2, 3] hydraulic mobilization, [4, 5] and electro-osmotically-driven mobilization.[6, 7]

The BioMark 5-component mixture of pI markers can be used for external standardization, or for determination of the suitability of a CE system or capillary for isoelectric focusing. The mixture should be diluted 1:100 in a suitable ampholyte solution (*e.g.* 2% w/v Bio-Lyte® 3/10 ampholytes) for detection at 280 nm.

Individual BioMark pI markers can be used for internal standardization or combined to prepare a custom blend for external standardization. For internal standardization, each marker should be added to the protein + ampholyte mixture at a final concentration of 1% (v/v). To prepare a custom mixture of markers, each marker should be added to a suitable ampholyte solution at a final concentration of 1% (v/v).

BioMark pI markers are stable at ambient temperature, but for long term storage, a temperature of 4 °C is recommended.

Analysis Conditions for Capillary Isoelectric Focusing using Ion Addition Mobilization

The separation shown in Figure 1 was obtained using the BioFocus® automated capillary electrophoresis system with the following separation conditions; pI markers were mixed with Bio-Lyte 3/10 ampholytes at a final concentration of 2% ampholytes.

Capillary	24 cm x 50 µm, coated with linear polyacrylamide or equivalent
Injection	20 sec at 100 psi
Polarity (outlet)	positive (inlet) to negative
Focusing anolyte	20 mM phosphoric acid
Focusing catholyte	40 mM sodium hydroxide
Focusing voltage	15 kV
Focusing time	240 sec
Mobilization anolyte	20 mM phosphoric acid
Mobilization catholyte	zwitterion solution
Detection wavelength	280 nm
Capillary temperature	27 °C
Autosampler temperature	20 °C
Analysis time	30 min

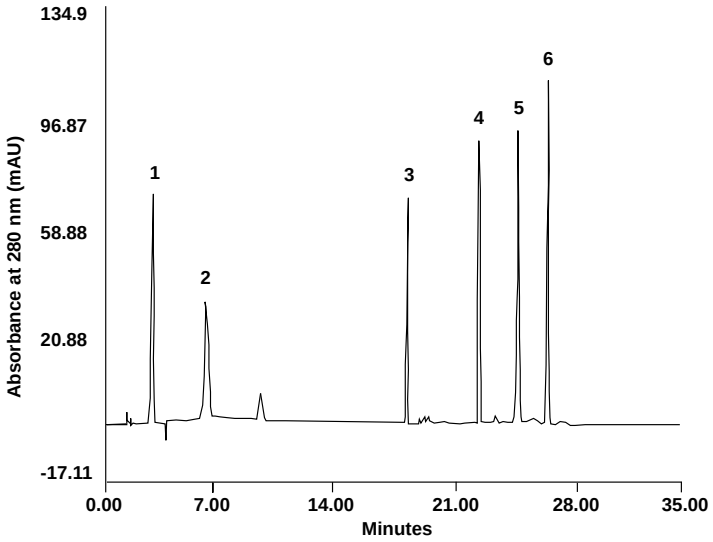

Fig. 1. Separation of the 5-component BioMark synthetic pI marker mixture. The mixture was separated on the BioFocus 3000 CE system using ion addition mobilization with conditions as described in the text. Peak identities: (1) focusing peak; (2) pI 10.4 marker; (3) pI 8.4 marker; (4) pI 7.4 marker, (5) pI 6.4 marker; (6) pI 5.3 marker.

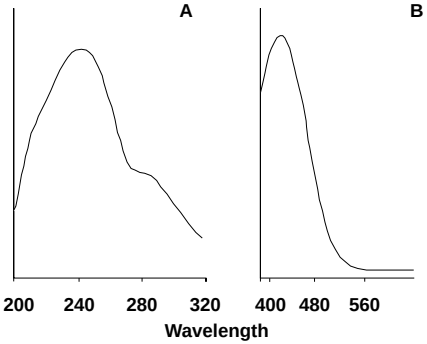

Fig. 2. Spectra of BioMark synthetic pI markers. (A) pI 5.3, UV spectrum; (B) pI 5.3, visible spectrum; (C) pI 6.4, UV spectrum; (D) pI 6.4, visible spectrum; (E) pI 7.4, UV spectrum, (F), pI 7.4, visible spectrum; (G) pI 8.4, UV spectrum; (H) pI 8.4, visible spectrum; (I) pI 10.4, UV spectrum; (J), pI 10.4, visible spectrum. The marker mixture was separated on the BioFocus CE system using ion addition mobilization with conditions described in the text. Spectra were obtained by scanning from 200–320 nm or 380–700 nm during mobilization.